

CLAIMS

What is claimed is:

1. A method of screening candidate substances for an ability to modulate phosphatidylserine exposure on the surface of a cell, the method
5 comprising:
 - (a) establishing a test sample comprising a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide;
 - (b) administering a candidate substance to the test sample; and
 - (c) measuring the interaction, effect, or combination thereof, of the
10 candidate substance on the test sample to thereby determine the ability of the candidate substance to modulate phosphatidylserine exposure on the surface of the cell.
2. The method of claim 1, wherein the candidate substance is a
15 candidate polypeptide, an antibody, a nucleic acid, or a chemical compound.
3. The method of claim 2, further comprising isolating a gene encoding the candidate polypeptide.
- 20 4. The method of claim 1, wherein the test sample comprises a nucleic acid molecule encoding a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide.
5. The method of claim 1, wherein the platelet voltage dependent
25 calcium channel (VDCC) α_1 subunit polypeptide is contained within a cell in cell culture.
6. A recombinant cell line suitable for use in the method of claim 5.
- 30 7. The method of claim 1, wherein the measuring comprises:
 - (a) determining a first level of phosphatidylserine exposure on a surface of the test sample before the administering step (b);
 - (b) determining a second level of phosphatidylserine exposure on

the surface of the test sample after the administering step (b);
and

- (c) comparing the first level and the second level, wherein a difference between the first level and the second level is indicative of the ability of the candidate substance to modulate phosphatidylserine exposure on the surface of the cell.

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8. The method of claim 7, wherein the determining comprises:

- (a) contacting the test sample with an antibody comprising a detectable moiety, wherein the antibody binds to phosphatidylserine; and

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- (b) quantitating an amount of the antibody bound to the test sample, wherein the amount of the antibody bound to the test sample is indicative of an amount of phosphatidylserine on the surface of the test sample.

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9. The method of claim 8, wherein the detectable moiety is selected from the group consisting of a radiolabel, a fluorescent label, a chemiluminescent label, and an enzyme.

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10. The method of claim 8, wherein the quantitating is by a method selected from the group consisting of radioactive detection, fluorescence detection, chemiluminescent detection, calorimetric detection, Western blotting, immunoprecipitation, and fluorescence-activating cell sorting (FACS).

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11. The method of claim 1, wherein the interaction, effect, or combination thereof, of the candidate substance on the test sample results in an increase in phosphatidylserine exposure on the surface of the cell.

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12. The method of claim 1, wherein the interaction, effect, or combination thereof, of the candidate substance on the test sample results in a decrease in phosphatidylserine exposure on the surface of the cell.

13. A method of screening candidate substances for an ability to modulate phosphatidylserine exposure on a surface of a cell, the method comprising:

- 5 (a) establishing a test sample comprising a modulatable transcriptional regulatory sequence of a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide-encoding gene and a reporter gene which is capable of producing a detectable signal;
- 10 (b) administering a candidate substance to the test sample; and
- (c) measuring the detectable signal produced as a result of an interaction between the candidate substance and the test sample, wherein a candidate substance as a modulator of phosphatidylserine exposure on a surface of a cell is based on
15 the amount of signal produced in relation to a control sample.

14. The method of 13, wherein the reporter gene encodes a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide.

20 15. A method of modulating phosphatidylserine exposure on a surface of a cell, the method comprising administering to the cell an effective amount of a substance capable of modulating the activity of a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide in the cell to thereby modulate phosphatidylserine exposure on the surface of the cell.

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16. The method of claim 15, wherein the cell is a platelet or a megakaryocyte.

17. The method of claim 15, wherein the cell comprises a cell in a
30 vertebrate subject.

18. The method of claim 17, wherein the vertebrate subject is a mammal.

19. The method of claim 15, wherein the administering further comprises administering an effective amount of a substance that modulates expression of a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide-encoding nucleic acid molecule in the cell.

20. The method of claim 15, wherein the substance capable of modulating the activity of a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide in the cell comprises an anti-platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide antibody, a polypeptide, a chemical compound, or a nucleic acid.

21. The method of claim 20, wherein the nucleic acid comprises an antisense oligonucleotide.

22. The method of claim 20, wherein the polypeptide or chemical compound comprises a ligand for a modulatable transcriptional regulatory sequence of a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide-encoding nucleic acid molecule.

23. A pharmaceutical composition comprising a therapeutically effective amount of a modulator of phosphatidylserine exposure on a surface of a cell and a pharmaceutically acceptable diluent or vehicle.

24. The pharmaceutical composition of claim 23, wherein the modulator of phosphatidylserine exposure on a surface of a cell binds a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide, or a fragment or derivative thereof.

25. A method for modulating phosphatidylserine exposure on a surface of a cell, the method comprising introducing to the cell a construct comprising a nucleic acid sequence encoding a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide operatively linked to a

promoter, wherein production of the platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide in the cell results in modulation of phosphatidylserine exposure on a surface of the cell.

5 26. The method of claim 25, wherein the construct further comprises a vector selected from the group consisting of a plasmid vector and a viral vector.

 27. The method of claim 25, wherein the construct further comprises
10 a liposome complex.

 28. The method of claim 25, wherein the cell is a platelet or a megakaryocyte.

15 29. The method of claim 25, wherein the cell comprises a cell in a vertebrate subject.

 30. The method of claim 29, wherein the vertebrate subject is a mammal.

20 31. A method for modulating thrombosis in a subject, the method comprising administering to the subject an effective amount of a substance capable of modulating the activity of a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide in the cell, wherein thrombosis in the
25 subject is modulated.

 32. The method of claim 31, wherein the subject is a mammal.

 33. The method of claim 32, wherein the mammal is a human.

30 34. The method of claim 31, wherein the administering further comprises administering an effective amount of a substance that modulates expression of a platelet voltage dependent calcium channel (VDCC) α_1

subunit polypeptide-encoding nucleic acid molecule in the cell.

35. The method of claim 34, wherein the substance that modulates expression of a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide-encoding nucleic acid molecule in the cell comprises an anti-platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide antibody, a polypeptide, a chemical compound, or a nucleic acid.

36. The method of claim 35, wherein the nucleic acid comprises an antisense oligonucleotide.

37. The method of claim 36, wherein the polypeptide or chemical compound comprises a ligand for a modulatable transcriptional regulatory sequence of a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide-encoding nucleic acid molecule.

38. A pharmaceutical composition comprising a therapeutically effective amount of a modulator of thrombosis and a pharmaceutically acceptable diluent or vehicle, wherein the modulator of thrombosis binds a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide, or a fragment or derivative thereof.

39. A method for modulating thrombosis in a subject, the method comprising introducing into a cell present within the subject a construct comprising a nucleic acid sequence encoding a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide operatively linked to a promoter, wherein production of the platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide in the cell results in modulation of thrombosis in the subject.

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40. The method of claim 39, wherein the construct further comprises a vector selected from the group consisting of a plasmid vector and a viral vector.

41. The method of claim 39, wherein the construct further comprises a liposome complex.
- 5 42. The method of claim 39, wherein the cell is a platelet or a megakaryocyte.
43. The method of claim 39, wherein the subject is a mammal.
- 10 44. The method of claim 43, wherein the mammal is a human.
45. A method of screening candidate substances for an ability to modulate platelet voltage dependent calcium channel (VDCC) α_1 subunit biological activity, the method comprising:
- 15 (a) establishing a test sample comprising a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide;
- (b) administering a candidate substance to the test sample; and
- (c) measuring the interaction, effect, or combination thereof, of the candidate substance on the test sample to thereby determine
- 20 the ability of the candidate substance to modulate platelet voltage dependent calcium channel (VDCC) α_1 subunit biological activity.
46. The method of claim 45, wherein the candidate substance is a
- 25 candidate polypeptide, an antibody, a nucleic acid, or a chemical compound.
47. The method of claim 46, further comprising the step of purifying and isolating a gene encoding the candidate polypeptide.
- 30 48. The method of claim 45, wherein the test sample comprises a nucleic acid molecule encoding a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide.

49. The method of claim 45, wherein the platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide is contained within cells in cell culture.
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50. A recombinant cell line suitable for use in the method of claim 49.
51. The method of claim 45, wherein the measuring comprises:
- 10 (a) determining a first level of phosphatidylserine exposure in the test sample before the administering step (b);
- (b) determining a second level of phosphatidylserine exposure in the test sample after the administering step (b); and
- (c) comparing the first level and the second level, wherein a
- 15 difference between the first level and the second level is indicative of the ability of the candidate substance to modulate platelet voltage dependent calcium channel (VDCC) α_1 subunit biological activity.
- 20 52. The method of claim 51, wherein the determining comprises:
- (a) contacting the test sample with an antibody comprising a detectable moiety, wherein the antibody binds to phosphatidylserine; and
- (b) quantitating an amount of the antibody bound to the test sample,
- 25 wherein the amount of the antibody bound to the test sample is indicative of the ability of the candidate substance to modulate platelet voltage dependent calcium channel (VDCC) α_1 subunit biological activity.
- 30 53. The method of claim 52, wherein the detectable moiety is selected from the group consisting of a radiolabel, a fluorescent label, a chemiluminescent label, and an enzyme.

54. The method of claim 52, wherein the quantitating is by a method chosen from the group consisting of radioactive detection, fluorescence detection, chemiluminescent detection, calorimetric detection, Western blotting, immunoprecipitation, and fluorescence-activating cell sorting (FACS).

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55. A method of screening candidate substances for an ability to modulate platelet voltage dependent calcium channel (VDCC) α_1 subunit biological activity, the method comprising:

- 10 (a) establishing a test sample comprising a modulatable transcriptional regulatory sequence of a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide-encoding gene and a reporter gene which is capable of producing a detectable signal;
- (b) administering a candidate substance to the test sample; and
- 15 (c) measuring the detectable signal produced as a result of an interaction between the candidate substance and the test sample, wherein a candidate substance as a modulator of platelet voltage dependent calcium channel (VDCC) α_1 subunit biological activity is based on the amount of signal produced in
- 20 relation to a control sample.

56. The method of 55, wherein the reporter gene encodes a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide.

25 57. A method of modulating platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide biological activity in a cell, the method comprising administering to the cell an effective amount of a substance capable of modulating activity of a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide in the cell to thereby modulate platelet voltage

30 dependent calcium channel (VDCC) α_1 subunit polypeptide biological activity in the cell.

58. The method of claim 57, wherein the cell is a platelet or a megakaryocyte.
59. The method of claim 58, wherein the cell comprises a cell in a vertebrate subject.
60. The method of claim 59, wherein the vertebrate subject is a mammal.
61. The method of claim 57, wherein the administering further comprises administering an effective amount of a substance that modulates expression of a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide-encoding nucleic acid molecule in the cell.
62. The method of claim 57, wherein the substance that modulates the platelet voltage dependent calcium channel (VDCC) α_1 subunit biological activity comprises an anti-platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide antibody, a polypeptide, a chemical compound, or a nucleic acid.
63. The method of claim 62, wherein the nucleic acid comprises an antisense oligonucleotide.
64. The method of claim 62, wherein the polypeptide or chemical compound comprises a ligand for a modulatable transcriptional regulatory sequence of a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide-encoding nucleic acid molecule.
65. A pharmaceutical composition comprising a therapeutically effective amount of a modulator of a biological activity of a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide and a pharmaceutically acceptable diluent or vehicle.

66. The pharmaceutical composition of claim 65, wherein the modulator binds a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide, or a fragment or derivative thereof.

5 67. A method for modulating calcium transport in a cell, the method comprising introducing to the cell a construct comprising a nucleic acid sequence encoding a platelet voltage dependent calcium channel (VDCC) α_1 subunit polypeptide operatively linked to a promoter, wherein production of the platelet voltage dependent calcium channel (VDCC) α_1 subunit
10 polypeptide in the cell results in modulation of calcium transport.

68. The method of claim 67, wherein the construct further comprises a vector selected from the group consisting of a plasmid vector or a viral
15 vector.

69. The method of claim 67, wherein the construct further comprises a liposome complex.

70. The method of claim 67, wherein the cell is a platelet or a
20 megakaryocyte.

71. The method of claim 67, wherein the cell comprises a cell in a vertebrate subject.

25 72. The method of claim 71, wherein the vertebrate subject is a mammal.

73. A method of modulating apoptosis in a cell selected from the group consisting of a platelet and a megakaryocyte and comprising a voltage
30 dependent calcium channel (VDCC) α_1 subunit polypeptide, the method comprising:

- (a) identifying a candidate substance that modulates the exposure of phosphatidylserine on a surface of the platelet or

megakaryocyte ; and

- (b) contacting the cell with the candidate substance identified in step (a), whereby apoptosis in the cell is modulated.

5 74. The method of claim 73, wherein the exposure of phosphatidylserine on a surface of the cell is increased, thereby enhancing apoptosis in the cell.

10 75. The method of claim 73, wherein the exposure of phosphatidylserine on a surface of the cell is decreased, thereby inhibiting apoptosis in the cell.

76. The method of claim 73, wherein the cell is a platelet.

15 77. A method of increasing a storage life of a cell selected from the group consisting of a platelet and a megakaryocyte, the method comprising:

- (a) identifying a candidate substance that decreases the exposure of phosphatidylserine on the surface of the cell; and
 - (b) contacting the cell with the candidate substance identified in
- 20 step (a), whereby the storage life of the cell is increased.

78. The method of claim 77, wherein the cell is a platelet.